FORM PTO-1390 (Modified) (REV 11-2000) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR
220037US0PCT	10/070430

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/EP00/07679	3 August 2000	16 September 1999

TITLE OF INVENTION

FORMULATIONS FOR PARENTERAL USE OF ESTRAMUSTINE PHOSPHATE WITH IMPROVED PHARMACOLOGICAL PROPERTIES

APPLICANT(S) FOR DO/EO/US

MUGGETTI Lorena et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include itens (5), (6), (9) and (24) indicated below.
4. ☒ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   b. ☒ has been communicated by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☐ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☒ A copy of the International Search Report (PCT/ISA/210).

Items 13 to 20 below concern document(s) or information included:

13. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☒ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
20. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
21. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
22. ☐ Certificate of Mailing by Express Mail
23. ☒ Other items or information:

PCT/IB/304
PCT/IB/308
Form PTO-1449
Request for Priority

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
10/070430	PCT/EP00/07679	220037US0PCT

24. The following fees are submitted:.		CALCULATIONS PTO USE ONLY	
BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :			
☐ Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO	**$1040.00**		
☒ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO	**$890.00**		
☐ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO	**$740.00**		
☐ International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4)	**$710.00**		
☐ International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4)	**$100.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =		**$890.00**	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).		**$0.00**	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	24 - 20 =	4	x $18.00	**$72.00**	
Independent claims	5 - 3 =	2	x $84.00	**$168.00**	
Multiple Dependent Claims **(check if applicable)**.			☐	**$0.00**	
TOTAL OF ABOVE CALCULATIONS =				**$1,130.00**	
☐ Applicant claims small entity status. See 37 CFR 1.27). The fees indicated above are reduced by 1/2.				**$0.00**	
SUBTOTAL =				**$1,130.00**	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). +				**$0.00**	
TOTAL NATIONAL FEE =				**$1,130.00**	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).** ☐				**$0.00**	
TOTAL FEES ENCLOSED =				**$1,130.00**	
				Amount to be: refunded	$
				charged	$

a. ☒ A check in the amount of **$1,130.00** to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **15-0030** A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

22850

Surinder Sachar
Registration No. 34,423

Surinder Sachar
SIGNATURE

Norman F. Oblon
NAME

24,618
REGISTRATION NUMBER

March 18 2002
DATE

220037US-0PCT

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF: :

LORENA MUGGETTI ET AL. :

SERIAL NO: NEW U.S. PCT APPLN. : ATTN: APPLICATION BRANCH
(Based on PCT/EP00/07679)

FILED: HEREWITH :

FOR: FORMULATIONS FOR PARENTERAL USE OF ESTRAMUSTINE PHOSPHATE WITH IMPROVED PHARMACOLOGICAL PROPERTIES

PRELIMINARY AMENDMENT

ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

SIR:

Prior to examination on the merits, please amend the above-identified application as follows.

IN THE CLAIMS

Please amend the claims as shown on the marked-up copy following this amendment to read as follows.

3. (Amended) A formulation according to claim 1 which is in single infusion dosage form comprising at least 1300 mg, of the estramustine phosphate.

4. (Amended) A formulation according to claim 1 which is in single infusion dosage form comprising at least 950 mg/m^2, of the estramustine phosphate.

5. (Amended) A formulation according to claim 1 wherein the sulfoalkyl ether cyclodextrin is a straight or branched C_1-C_6 sulfoalkyl ether cyclodextrin.

7. (Amended) A formulation according to claim 1 for intravenous use.

8. (Amended) A formulation according to claim 1 wherein the estramustine phosphate is in the form of a pharmaceutically acceptable salt for intravenous use.

10. (Amended) A formulation according to claim 1 for use in the treatment of cancer.

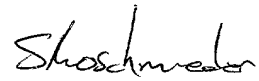
16. (Amended) A product according to claim 14 wherein the chemotherapeutic agent is selected from taxane, taxane derivatives, CPT-11, camptothecin and derivatives thereof, doxorubicin, idarubicin, epirubicin, etoposide, navelbine, vinblastine, carboplatin, cisplatin, Sugen SU 6668 and Sugen SU 5416.

REMARKS

Claims 1-24 are active in the present application. Claims 3-5, 7-8, 10, 16, have been amended to remove multiple dependencies. No new matter is added. An action on the merits and allowance of claims is solicited.

Respectfully submitted,

OBLON, SPIVAK, McCLELLAND,
MAIER & NEUSTADT, P.C.



Norman F. Oblon
Attorney of Record
Registration No. 24,618

Stefan U. Koschmieder, Ph.D.
Registration No. 50,238

22850

(703) 413-3000
NFO/DJP/smi
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Marked-Up Copy
Serial No:

Amendment Filed on:
3-18-2002

IN THE CLAIMS

Please amend the claims as follows.

--3. (Amended) A formulation according to claim 1 [or 2] which is in single infusion dosage form comprising at least 1300 mg, of the estramustine phosphate.

4. (Amended) A formulation according to [any one of the preceding claims] claim 1 which is in single infusion dosage form comprising at least 950 mg/m^2, of the estramustine phosphate.

5. (Amended) A formulation according to [any on of the preceding claims] claim 1 wherein the sulfoalkyl ether cyclodextrin is a straight or branched C_1-C_6 sulfoalkyl ether cyclodextrin.

7. (Amended) A formulation according to [any one of the preceding claims] claim 1 for intravenous use.

8. (Amended) A formulation according to [any one of the preceding claims] claim 1 wherein the estramustine phosphate is in the form of a pharmaceutically acceptable salt for intravenous use.

10. (Amended) A formulation according to [any one of the preceding claims] claim 1 for use in the treatment of cancer.

16. (Amended) A product according to claim 14 [or 15] wherein the chemotherapeutic agent is selected from taxane, taxane derivatives, CPT-11, camptothecin

and derivatives thereof, doxorubicin, idarubicin, epirubicin, etoposide, navelbine, vinblastine, carboplatin, cisplatin, Sugen SU 6668 and Sugen SU 5416.--

DOCKET NO.: 220037US0PCT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF: Lorena MUGGETTI, et al.
SERIAL NO.: NEW U.S. PCT APPLICATION
FILED: HEREWITH
INTERNATIONAL APPLICATION NO.: PCT/EP00/07679
INTERNATIONAL FILING DATE: August 3, 2000
FOR: FORMULATIONS FOR PARENTERAL USE OF ESTRAMUSTINE PHOSPHATE WITH IMPROVED PHARMACOLOGICAL PROPERTIES

REQUEST FOR PRIORITY UNDER 35 U.S.C. 119 AND THE INTERNATIONAL CONVENTION

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

In the matter of the above-identified application for patent, notice is hereby given that the applicant claims as priority:

COUNTRY	APPLICATION NO	DAY/MONTH/YEAR
Great Britain	9921954.5	16 September 1999

Certified copies of the corresponding Convention application(s) were submitted to the International Bureau in PCT Application No. PCT/EP00/07679. Receipt of the certified copy(s) by the International Bureau in a timely manner under PCT Rule 17.1(a) has been acknowledged as evidenced by the attached PCT/IB/304.

Respectfully submitted,
OBLON, SPIVAK, McCLELLAND,
MAIER & NEUSTADT, P.C.

Norman F. Oblon
Attorney of Record
Registration No. 24,618
Surinder Sachar
Registration No. 34,423

22850
(703) 413-3000
Fax No. (703) 413-2220
(OSMMN 1/97)

FORMULATIONS FOR PARENTERAL USE OF ESTRAMUSTINE PHOSPHATE WITH IMPROVED PHARMACOLOGICAL PROPERTIES

The present invention relates to pharmaceutical formulations of estramustine phosphate for parenteral use with improved pharmacological properties and, more particularly, to formulations of estramustine phosphate for parenteral use further comprising sulfoalkyl ether cyclodextrins and human albumin.

Estramustine phosphate (The Merck Index, XII Ed., No. 3749, 1996) is an estradiol-17β-phosphate derivative widely known in the art as antitumor agent, currently used in the treatment of advanced adenocarcinoma of the prostate.
The drug is usually administered orally, preferably at a dose of 10-15 mg/kg/day. Intravenous administration, however, is also adopted in some particular cases.
For example, initial intravenous administration of estramustine phosphate, followed by oral administration, has been reported at dosages paralleling the oral administration for the drug, i.e. 300-600 mg daily given intravenously and usually repetitively over for several consecutive days (see, for a reference, British Journal of Urology, 1977, 49, 73-79; J. Urol.108:303-306, 1972; Eur. Clin. Pharmacol. 26(1), 113-119, 1984; Eur. Urol. 1990, 17, 216-218).

Estramustine phosphate as well as other well-known cytotoxic compounds used in antitumor therapy are known to cause, or potentially cause, vascular damages at the site of injection when parenterally, in particular intravenously, administered.
As an example, studies in patients treated with estramustine phosphate administered as a slow intravenous injection or as a bolus, at 300 mg/day, revealed

thrombophlebitis and local irritations at the peripheral intravenous injection sites.
These drawbacks are considered major limitations for the intravenous administration of estramustine phosphate, thus requiring, in many patients, the establishment of central line administration or, in some cases, even discontinuation of the treatment.

With the aim of minimising the unwanted effects associated with the intravenous administration of cytotoxic agents, a few means are reported in the art.
Among them is the use of cyclodextrins, for instance hydroxypropyl-cyclodextrin, in the preparation of formulations for parenteral administration of cytotoxic known to cause ulcerative lesions. See, for a reference, US patent No. 5,804,568 in the name of Supergen Inc.
Cyclodextrin derivatives such as sulfoalkyl ether cyclodextrins are known in the art as solubilizing agents for insoluble or poorly soluble drugs (see, for a reference, US 5,134,127 in the name of the University of Kansas).
Also known in the art are formulations for the intravenous administration of estramustine phosphate containing human albumin, reported to be characterised by fewer local side-effects upon injection of the active (see, for a reference, H. Schutz et al.; Krankenhauspharmazie, II year, issue No. 3, 1988).

In this respect, we found formulations for parenteral use comprising estramustine phosphate together with sulfoalkyl ether cyclodextrins and human albumin which, unexpectedly, resulted to achieve optimal protection from side-effects associated with estramustine administration.

It is therefore the object of the present invention a formulation for parenteral use comprising estramustine

phosphate in admixture with a sulfoalkyl ether cyclodextrin and human albumin.

Once administered intravenously to patients, the formulations object of the present invention do not provoke ulcerative damages, nor thrombophlebitis, at the site of injection.

Very interestingly, the estramustine phosphate formulations of the invention result to be endowed with unexpected pharmacological properties, expressed in terms of toxicity at the site of injection, markedly improved with respect to formulations containing, as a single protective excipient, a sulfoalkyl ether cyclodextrin or, alternatively, human albumin.

In the present invention, unless otherwise specified, with the term formulation comprising estramustine phosphate, as the active ingredient, we intend any formulation comprising estramustine phosphate either in the acid form or as a pharmaceutically acceptable salt for parenteral administration such as, for instance, a salt with a basic amino acid or with N-methyl glucamine, otherwise referred to as meglumine.

Preferably, estramustine phosphate is in the form of its meglumine salt.

With the term sulfoalkyl ether cyclodextrin we refer to any cyclodextrin of the above type wherein alkyl stands for straight or branched C_1-C_6 alkyl group such as methyl, ethyl, n.propyl, isopropyl, n.butyl, isobutyl, sec-butyl, tert-butyl, n.pentyl, n.hexyl and the like.

Preferably, the formulation of the present invention comprises estramustine phosphate in admixture with sulfobutyl ether ß-cyclodextrin.

According to a preferred embodiment of the invention, the weight ratio between estramustine phosphate and sulfoalkyl

ether cyclodextrin is comprised from about 1:0.5 to about 1:5, respectively.
However, higher amounts of sulfoalkyl ether cyclodextrin with respect to the active are sill effective and hence comprised within the scope of the present invention.

According to another preferred embodiment of the invention, the above formulations are advantageously used for intravenous use.
As such, these formulations of the invention can be administered to patients either as a slow injection, e.g. over about 30 minutes to about 3 hours, or as a bolus injection, also referred to as IV (intravenous) push.

In another preferred embodiment of the invention either
(i) the estramustine phosphate is in lyophilised form and the parenterally acceptable carrier or diluent is a physiological solution for parenteral use containing the sulfoalkyl ether cyclodextrin and the human albumin, or
(ii) the estramustine phosphate and sulfoalkyl ether cyclodextrin are in lyophilised form and the parenterally acceptable carrier or diluent is a physiological solution for parenteral use containing the human albumin.

The invention also provides a product which comprises estramustine phosphate in lyophilised form and a physiological solution for parenteral use containing human albumin.

The formulations of the invention also provide a very advantageous method for delivering estramustine phosphate intravenously, even when high doses of the active are needed.
It is therefore a further object of the invention a formulation for parenteral use comprising estramustine

phosphate, as a single infusion dosage of the active exceeding 1300 mg, in admixture with a sulfoalkyl ether cyclodextrin and human albumin.

According to another preferred embodiment of the invention, it is further provided a formulation for parenteral use comprising estramustine phosphate, as a single infusion dosage of the active exceeding 950 mg/m^2, in admixture with a sulfoalkyl ether cyclodextrin and human albumin.

The formulations object of the present invention allow the administration of the active either as a single agent or, alternatively, in combination with known anticancer treatments such as radiation therapy or chemotherapy regimen in combination with cytostatic or cytotoxic agents, antibiotic-type agents, alkylating agents, antimetabolite agents, hormonal agents, e.g. aromatase inhibitors, immunological agents, interferon-type agents, cyclooxygenase inhibitors (e.g. COX-2 inhibitors), metallomatrixprotease inhibitors, telomerase inhibitors, tyrosine kinase inhibitors, anti-growth factor receptor agents, anti-HER agents, anti-EGFR agents, anti-angiogenesis agents, farnesyl transferase inhibitors, ras-raf signal transduction pathway inhibitors, cell cycle inhibitors, other cdks inhibitors, tubulin binding agents, topoisomerase I inhibitors, topoisomerase II inhibitors, and the like.

As an example, the above formulations can be administered in combination with one or more chemotherapeutic agents, optionally within liposomal formulations thereof.
Examples of chemotherapeutic agents are, for instance, taxane, taxane derivatives, CPT-11, camptothecin and derivatives thereof, anthracycline glycosides, e.g. doxorubicin, idarubicin or epirubicin, etoposide, navelbine, vinblastine, carboplatin, cisplatin and the like, optionally within liposomal formulations thereof.

In addition, the above formulations can be also administered in combination with protein kinase inhibitors such as, for instance, the indolinone derivatives disclosed by Sugen in the international patent applications WO 96/40116 and WO 99/61422, which are herewith incorporated by reference.

In this respect, the formulations object of the invention can be preferably administered in combination with 3-[4-(2-carboxyethyl-3,5-dimethylpyrrol-2-yl)methylidenyl]-2-indolinone and 3[(2,4-dimethylpyrrol-5-yl)methylidenyl]-2-indolinone, better known as Sugen SU 6668 and SU 5416, respectively.

The formulations of the invention may be administered sequentially with known anticancer agents when a combination formulation is inappropriate.

Therefore, it is a further object of the present invention a product containing a formulation for parenteral use of estramustine phosphate in admixture with a sulfoalkyl ether cyclodextrin and human albumin and one or more chemotherapeutic agents, as a combined preparation for simultaneous, separate or sequential use in anticancer therapy.

Toxicology

To study the local irritant effects of estramustine phosphate after repeated intravenous administrations to rats, in comparison to a formulation of estramustine phosphate according to the present invention, the active was dissolved in different vehicles such as water solution for injection and water solution for injection further containing sulfobutyl ether ß-cyclodextrin and human albumin. In addition, formulations of estramustine phosphate in admixture with sulfobutyl ether ß-cyclodextrin only or, alternatively, with human albumin only, were used for comparison.

In particular, the following water for injection solutions wherein either the active estramustine phosphate (therein referred to as EMP) as well as the excipients sulfobutyl ether ß-cyclodextrin and human albumin (therein referred to as SBECD and HA, respectively) are expressed in terms of weight ratios, were prepared and tested:

(a)	**negative control:**	**water for injection;**
(b)	**positive control:**	**EMP;**
(c)	**comparison:**	**EMP:SBECD=1:1;**
(d)	**comparison:**	**EMP:HA=1:0.21;**
(e)	**tested solution:**	**EMP:SBECD:HA=1:1:0.21;**

Male Sprague-Dawley rats were used because of their acceptance as a predictor of toxic change in man. The rats were 6 weeks old at the start of the study.

Estramustine phosphate, in the form of meglumine salt, was administered to groups of rats as a repeated intravenous injection during 3 days. Rats were then sacrificed: a half of the rats at the fourth day and a half at the fifth day.

The dose level of estramustine phosphate, in all the different tested solutions, was of 150 mg/kg/day.

Clinical observations were recorded daily. Thrombophlebitic side effects resulted in a dark bluish/blackish coloration of the tail during the treatment period.

A score system based on tail coloration and its extension was used to evaluate the different tested formulations.

The score system considered estramustine phosphate water solution **(b)** as the positive control (i.e. marked toxicity). Water for injection **(a)** was administered to the control group as negative control (i.e. no toxicity signs).

Histological evaluation was carried out on the tail of the rats treated with the composition of the invention.

Estramustine phosphate in a water solution **(b)** induced, at the used dose, local irritant effects at the injection site after the first administration and marked toxicity signs at the end of the experiment.

Likewise, toxicity signs at the injection site were also observed for the comparison **(c)** and **(d)** EMP solutions containing SBECD or HA, as the sole excipients.

On the contrary, no toxicity was observed with the formulation of the invention containing sulfobutyl ether ß-cyclodextrin and human albumin **(e)**.

Moreover, histological evaluation of the tail of the rats treated with the formulation **(e)** did not reveal any damage when compared to the tails of the control group.

In addition, the synergistic protective effect exerted by combining both SBECD and HA excipients, as per the invention, can clearly be evidenced by considering the formulation of the invention **(e)** in comparison to the formulations **(c)** and **(d)** wherein each single excipient is present in the same amount with respect to the active.

It was thus concluded that estramustine phosphate in a water solution containing sulfoalkyl ether cyclodextrin and human albumin, according to the present invention, induced markedly less local irritant effects when compared with a water solution of estramustine phosphate itself.

Even more surprisingly, the formulation of the invention produced less local irritant effects also in comparison to analogous solutions of estramustine phosphate containing sulfoalkyl ether cyclodextrin only or human albumin only.

One particularly preferred schedule for administering the formulation of estramustine phosphate according to the invention is a single infusion given once weekly to a maximal dose of 4000 mg or 3500 mg/m^2.

Another preferred schedule is the administration of a single drug infusion once every two to four weeks.

One schedule may be preferred over another in consideration of schedules with other optional concomitant therapy. These schedules may repeat in serial or as repetitive fashion.

The formulations of the present invention are useful in antitumor therapy, particularly in the treatment of prostate cancer, breast cancer, melanoma, lung cancer, pancreatic cancer, colorectal cancer, ovarian cancer and cancers of the brain.

The formulations object of the present invention are prepared according to conventional techniques adopted in the preparation of pharmaceutical forms for parenteral use. Typically, a proper amount of estramustine phosphate, either as a dry powder or into a lyophilised form, is dissolved in a pharmaceutically acceptable solution for parenteral use and then admixed with a proper amount of a sulfoalkyl ether cyclodextrin, for instance sulfobutyl ether ß-cyclodextrin.
The above solution is then admixed with a proper amount of human albumin, either as a dry powder or as a commercially available solution, e.g. human albumin 25%, 20% or 5%, optionally properly diluted.
As an example, a proper amount of estramustine phosphate in the form of a suitable salt such as, for instance, N-methyl glucamine salt, is dissolved in a suitable amount of sterile water or aqueous dextrose solution, e.g. 5% dextrose in water for intravenous administration, and then admixed with a proper amount of powdered sulfobutyl ether ß-cyclodextrin.
The above admixture is subsequently added with a proper amount of human albumin, for instance as a dry powder, and subsequently stirred, sterilised and lyophilised according to conventional techniques.
From the foregoing it is clear to the man skilled in the art that each of the ingredients of the invention such as sulfoalkyl ether cyclodextrin and human albumin, each

independently as a powder or into a suitable solution, can be admixed in any order to the active, already dissolved into a proper solution or in the form of a dry powder.
Likewise, the formulations of the invention can also be prepared by admixing the active with the aforementioned ingredients already properly combined as above indicated.
The final freeze-dried formulation is then prepared and stored in vials for injection; the addition of a proper amount of sterile water or a physiological solution for parenteral use enables the preparation of the final formulation to be injected.

The above method is also suitable for preparing high dosages estramustine phosphate formulations whilst maintaining the desired weight ratio between the components.
The unit strength of the formulation to be injected depended on the concentration of the active in the solution itself and, of course, on the filling volume of the vials used to prepare the final formulation.

Additionally, the formulations of the present invention may optionally contain pharmaceutically acceptable excipients for parenteral administration such as, for instance, bulking agents, e.g. lactose or mannitol, pH buffering agents, anti-oxidant agents, preservative agents, tonicity adjusters and the like.

The following examples are herewith intended to better illustrate the present invention without representing any limitation to it.

Example 1

Preparation of estramustine phosphate N-methyl glucamine salt in admixture with sulfobutyl ether ß-cyclodextrin (estramustine phosphate:sulfobutyl ether ß-cyclodextrin=1:1 weight ratio)

300 mg of estramustine phosphate were weighed in a beaker and dispersed by means of magnetic stirring in 5 ml of water. 120.8 mg of N-methyl-glucamine were then added under stirring to the watery dispersion of the active and, after a few minutes, a clear solution was obtained. 312.5 mg of sulfobutyl ether ß-cyclodextrin were added, maintaining the solution under stirring till the solubilization was completed.

The solution obtained was then brought to the final volume of 10 ml with water so as to reach a final concentration of 30 mg/ml of estramustine phosphate and 31.25 mg/ml of sulfobutyl ether ß-cyclodextrin (1:1 weight ratio - 1:0.25 molar ratio respectively).

A solution prepared as previously described, properly sterilized by filtration, was tested for its local vein tolerability in rats.

Example 2

The formulation described in Example 1 was also prepared by solubilization of the commercially available Estracyt® freeze-dried formulation containing 300 mg/vial of the active. The reconstitution of the formulation was made using 10 ml of a 31.25 mg/ml sulfobutyl ether ß-cyclodextrin solution so as to obtain a final concentration of 30 mg/ml of estramustine phosphate and 31.25 mg/ml of cyclodextrin (1:1 weight ratio - 1:0.25 molar ratio respectively).

Example 3

Preparation of estramustine phosphate N-methyl glucamine salt in admixture with human albumin (estramustine phosphate:albumin=1:0.21 weight ratio)

300 mg of estramustine phosphate were weighed in a beaker and dispersed by means of magnetic stirring in 5 ml of water. 120.8 mg of N-methyl-glucamine were then added under stirring to the watery dispersion of the active and, after a few minutes, a clear solution was obtained. 0.250 ml of a

commercially available solution of human albumin at 25% concentration were added whilst maintaining the solution under stirring.

The obtained solution was then brought to the final volume of 10 ml with water so as to reach a final concentration of 30 mg/ml of estramustine phosphate and 6.25 mg/ml of human albumin (1:0.21 weight ratio respectively).

A solution prepared as previously described, properly sterilized by filtration, was tested for its local vein tolerability in rats.

Example 4

The formulation described in Example 3 was also prepared by solubilization of the commercially available Estracyt® freeze-dried formulation containing 300 mg/vial of the active. The reconstitution of the formulation was made by using 10 ml of a 6.25 mg/ml human albumin solution so as to obtain a final concentration of 30 mg/ml of estramustine phosphate and 6.25 mg/ml of human albumin (1:0.21 weight ratio respectively).

The albumin solution could be prepared either by dissolving in water a proper amount of human albumin as a dry powder or by properly diluting a commercially available human albumin solution.

Example 5

Preparation of estramustine phosphate N-methyl glucamine salt in admixture with sulfobutyl ether ß-cyclodextrin and human albumin (estramustine phosphate:sulfobutyl ether ß-cyclodextrin:albumin=1:1:0.21 weight ratio, respectively).

300 mg of estramustine phosphate were weighed in a beaker and dispersed by means of magnetic stirring in 5 ml of water. 120.8 mg of N-methyl-glucamine were then added under stirring to the watery dispersion of the active and, after a few minutes, a clear solution was obtained. 312.5 mg of sulfobutyl ether ß-cyclodextrin were added, maintaining the solution under stirring until complete dissolution.

0.250 ml of a commercially available solution of human albumin at 25% concentration were then added, maintaining the solution under stirring.

The solution was then brought to the final volume of 10 ml with water so as to reach a final concentration of 30 mg/ml of estramustine phosphate, 31.25 mg/ml of sulfobutyl ether ß-cyclodextrin and 6.25 mg/ml of human albumin. The weight ratio between the components of the solution were as follows: estramustine phosphate:sulfobutyl ether ß-cyclodextrin:human albumin 1:1:0.21 respectively.

A solution prepared as previously described, properly sterilized by filtration, was tested for its local vein tolerability in rats.

Example 6

The formulation described in Example 4 was also prepared by solubilization of the commercially available Estracyt® freeze-dried formulation containing 300 mg/vial of the active. The reconstitution of the formulation was made by using 10 ml of a solution containing 31.25 mg/ml of sulfobutyl ether ß-cyclodextrin and 6.25 mg/ml of human albumin so as to reach a final concentration of 30 mg/ml of the active. The weight ratio between the components of the solution were as follows: estramustine phosphate:sulfobutyl ether ß-cyclodextrin:human albumin 1:1:0.21 respectively.

CLAIMS

1. A pharmaceutical formulation which comprises a parenterally acceptable carrier or diluent, estramustine phosphate, a sulfoalkyl ether cyclodextrin and human albumin.

2. A formulation according to claim 1 wherein the weight ratio of estramustine phosphate to the sulfoalkyl ether cyclodextrin is from about 1:0.5 to about 1:5.

3. A formulation according to claim 1 or 2 which is in single infusion dosage form comprising at least 1300 mg, of the estramustine phosphate.

4. A formulation according to any one of the preceding claims which is in single infusion dosage form comprising at least 950 mg/m^2, of the estramustine phosphate.

5. A formulation according to any one of the preceding claims wherein the sulfoalkyl ether cyclodextrin is a straight or branched C_1-C_6 sulfoalkyl ether cyclodextrin.

6. A formulation according to claim 5 wherein the sulfoalkyl ether cyclodextrin is sulfobutyl ether ß-cyclodextrin.

7. A formulation according to any one of the preceding claims for intravenous use.

8. A formulation according to any one of the preceding claims wherein the estramustine phosphate is in the form of a pharmaceutically acceptable salt for intravenous use.

9. A formulation according to claim 8 wherein the estramustine phosphate is in the form of N-methyl glucamine salt.

10. A formulation according to any one of the preceding claims for use in the treatment of cancer.

11. A formulation as claimed in claim 10 wherein the cancer is prostate cancer, breast cancer, melanoma, lung cancer, pancreatic cancer, colorectal cancer, ovarian cancer or cancer of the brain.

12. A formulation according to claim 1 wherein the estramustine phosphate is in admixture with the sulfoalkyl ether cyclodextrin and the human albumin.

13. A formulation according to claim 1 wherein
(i) the estramustine phosphate is in lyophilised form and the parenterally acceptable carrier or diluent is a physiological solution containing the sulfoalkyl ether cyclodextrin and the human albumin, or
(ii) the estramustine phosphate and sulfoalkyl ether cyclodextrin are in lyophilised form and the parenterally acceptable carrier or diluent is a physiological solution containing the human albumin.

14. A product which comprises
(i) a pharmaceutical formulation which comprises a parenterally acceptable carrier or diluent and estramustine phosphate in admixture with a sulfoalkyl ether cyclodextrin and human albumin, and
(ii) one or more chemotherapeutic agents,
as a combined preparation for simultaneous, separate or sequential use in anticancer therapy.

15. A product according to claim 14 wherein the sulfoalkyl ether cyclodextrin is sulfobutyl ether ß-cyclodextrin.

16. A product according to claim 14 or 15 wherein the chemotherapeutic agent is selected from taxane, taxane derivatives, CPT-11, camptothecin and derivatives thereof, doxorubicin, idarubicin, epirubicin, etoposide, navelbine, vinblastine, carboplatin, cisplatin, Sugen SU 6668 and Sugen SU 5416.

17. A product according to claim 14 for intravenous use.

18. A product according to claim 14 for use in the treatment of prostate cancer, breast cancer, melanoma, lung cancer, pancreatic cancer, colorectal cancer, ovarian cancer or cancer of the brain.

19. A formulation as defined in claim 7 for use in suppressing or reducing the side-effects associated with the intravenous administration of estramustine phosphate and pharmaceutically acceptable salts thereof.

20. A formulation according to claim 19 wherein the side effects comprise ulcerative lesions and thrombophlebitis at the site of injection.

21. A product which comprises estramustine phosphate in lyophilised form and a physiological solution for parenteral use containing a sulfoalkyl ether cyclodextrin and human albumin.

22. A product which comprises estramustine phosphate and sulfoalkyl ether cyclodextrin in lyophilised form and a physiological solution for parenteral use containing human albumin.

23. Use, in the manufacture of a medicament for parenteral administration, of estramustine phosphate in admixture with a sulfoalkyl ether cyclodextrin and human albumin.

24. Use according to claim 23 wherein the medicament is for intravenous administration.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
22 March 2001 (22.03.2001)

PCT

(10) International Publication Number
WO 01/19338 A1

(51) International Patent Classification[7]: A61K 9/08, 47/40, 31/565, A61P 35/00, A61K 31/66, 47/42

(21) International Application Number: PCT/EP00/07679

(22) International Filing Date: 3 August 2000 (03.08.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
9921954.5 16 September 1999 (16.09.1999) GB

(71) Applicant *(for all designated States except US)*: **PHARMACIA & UPJOHN S.P.A.** [IT/IT]; Via Robert Koch, 1.2, I-20152 Milan (IT).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **MUGGETTI, Lorena** [IT/IT]; Via Trento, 38, I-20036 Meda (IT). **COLOMBO, Paolo** [IT/IT]; Via Ludovico Cavaleri, 3, I-20147 Milan (IT). **MARTINI, Alessandro** [IT/IT]; Via Desiderio da Settignano, 14, I-20149 Milan (IT). **BUZZI, Giovanni** [IT/IT]; Via F. Poggi, 14, I-20131 Milan (IT).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*
— *Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: FORMULATIONS FOR PARENTERAL USE OF ESTRAMUSTINE PHOSPHATE WITH IMPROVED PHARMACOLOGICAL PROPERTIES

(57) Abstract: A pharmaceutical formulation which comprises a parenterally acceptable carrier or diluent, estramustine phosphate, a sulfoalkyl ether cyclodextrin and human albumin. The formulation can be administered according to a combined chemotherapy regimen in association with one or more chemotherapeutic agents. The formulation also enables the estramustine phosphate to be administered with no side effects at the site of injection.

WO 01/19338 A1

Declaration and Power of Attorney for Patent Application
Dichiarazione e procura ai fini della domanda di brevetto

Italian Language Declaration

Il sottoscritto inventore dichiara che:

La propria residenza, recapito postale e cittadinanza corrispondono a quanto indicato in calce, sotto la propria firma.

Ritiene di essere il primo ed unico inventore originale (se viene elencato in calce un solo nominativo) o il coinventore primo ed originale (se è elencato più di un nominativo) del oggetto rivendicato e per il quale il sottoscritto presenta domanda di brevetto. La invenzione in questione è chiamata.

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Il sottoscritto dichiara in oltre di aver letto e compreso il contenuto della descrizione identificata in precedenza, rivendicazioni comprese, come modificati dall'eventuale modifica summenzionata.

Il sottoscritto riconosce l' obbligo di rivelare informazioni essenziali ai fini della determinazione della brevettabilità ai sensi del Titolo 37, Codice dei Regolamenti Federali, § 1.56.

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

FORMULATIONS FOR PARENTERAL USE OF ESTRAMUSTINE PHOSPHATE WITH IMPROVED PHARMACOLOGICAL PROPERTIES

the specification of which:

☐ is attached hereto.

☒ was filed on 3 AUGUST 2000

as United States Application Number or PCT International Application Number

PCT/EP00/07679 and was amended on

_____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56.

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Il sottoscritto rivendica con la presente la priorità prevista dal Titolo 35, Codice degli Stati Uniti, § 119(e)-(d) o § 365(b) in relazione a qualsiasi domanda o domande estere di brevetto o certificato di inventore, o dal Titolo 35, § 365(a) degli stessi Codice in relazione a qualsiasi domanda internazionale PCT nella quale è designato almeno un paese diverso dagli Stati Uniti, i suddetti domande e certifcati essendo elencati sotto, e, spuntando les seguenti caselle, ha anche identificato sotto qualsiasi domanda estera di brevetto o certificato di inventore, o domanda internazionale PCT, la cui data di deposito preceda quella dalla domanda per la quale è rivendicata la priorità.

I hereby claim foreign priority under Title 35, United States Code, § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States, listed below, and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s) (Domande Estere Anteriori)			Priority claimed Diritto di priorità rivendicato	
9921954.5 (Number) (Numero)	GB (Country) (Nazione)	16 SEPTEMBER 1999 (Day/Month/Year Filed) (Giorno/Mese/Anno di deposito)	XX Yes Sì	☐ No No
☐ (Number) (Numero)	☐ (Country) (Nazione)	☐ (Day/Month/Year Filed) (Giorno/Mese/Anno di deposito)	☐ Yes Sì	☐ No No

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I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below.

(Application No.) (Nº della domanda)	(Filing Date) (Data di deposito)	(Application No.) (Nº della domanda)	(Filing Date) (Data di deposito)

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I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s), or § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

(Application No.) (Nº della domanda)	(Filing Date) (Data di deposito)	(Status) (patented, pending, abandoned) (Stato) (concessione di brevetto, in corso di esame, abbandono)
(Application No.) (Nº della domanda)	(Filing Date) (Data di deposito)	(Status) (patented, pending, abandoned) (Stato) (concessione di brevetto, in corso di esame, abbandono)

Con la presente, il sottoscritto dichiara veritiere tutte le affermazioni contenute in questa domanda in relazione alle proprie conoscenze e di ritenere vere tutte le affermazioni o informazioni presentate. Dichiara inoltre che tali asserzioni sono state espresse nella piena consapevolezza che le dichiarazioni intenzionalmente false sono punibili con una poulte, l' incarcerazione o entrambe, ai sensi della Sezione 1001 del Titolo 18 del Codice degli Stati Uniti e che tali dichiarazioni entenzionalmente false possono mettere a repenfaglio la validità della domanda o di qualsiasi brevetto ruasciato in merito.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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PROCURA: Il sootscritto inventore nomina con la presente il seguente avvocato o avvocati e/o agente o agenti al fine di istruire questa pratica e di condurre tutte le operazione ad essa pertinenti presso l'Ufficio dei Brevetti e Marchi di Fabbrica: (Elencare il nome ed il numero di matricola).

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith: *(list name and registration number)*

022850

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Send Correspondence to:

022850

Telefonare a:
(Nome e numero telefonico)

Direct Telephone calls to: *(name and telephone number)*

(703) 413-3000

Nome e cognome dell'unico o del primo inventore	Full name of sole or first inventor LORENA MUGGETTI
Firma dell'inventore Data	Inventor's signature Date February 19, 2002
Residenza	Residence MEDA, MILAN, ITALY ITX
Cittadinanza	Citizenship ITALIAN
Recapito postale	Post Office Address VIA MEDA 38, 20036 MEDA, MILAN, ITALY
Nome e cognome dell'eventuale secondo coinventore	Full name of second joint inventor, if any PAOLO COLOMBO
Firma del secondo coinventore Data	Second inventor's signature Date February 19, 2002
Residenza	Residence MILAN, ITALY ITX
Cittadinanza	Citizenship ITALIAN
Recapito postale	Post Office Address VIA LUDOVICO CAVALERI 3, 20147 MILAN,
	ITALY

(Fornire le stesse informazioni e le firme del terzo e degli ulteriori coinventori.)

(Supply similar information and signature for third and subsequent joint inventors

Italian Language Declaration

Nome per intero di un eventuale terzo co-inventore	Full name of third joint inventor, if any ALESSANDRO MARTINI
Firma del Terzo Inventore Data	Third inventor's signature Date February 19, 2002
Residenza	Residence MILAN, ITALY
Cittadinanza	Citizenship ITALIAN
Recapito postale	Post Office Address VIA DESIDERIO DA SETTIGNANO 14, 20149
	MILAN, ITALY
Nome per intero di eventuale quarto co-inventore	Full name of fourth joint inventor, if any GIOVANNI BUZZI
Firma Quarto Inventore Data	Fourth inventor's signature Date February 19, 2002
Residenza	Residence MILAN, ITALY
Cittadinanza	Citizenship ITALIAN
Recapito postale	Post Office Address VIA E. POGGI 14, 20131 MILAN, ITALY
Nome per intero di un eventuale quinto co-inventore	Full name of fifth joint inventor, if any
Firma Quinto Inventore Data	Fifth inventor's signature Date
Residenza	Residence
Cittadinanza	Citizenship
Recapito postale	Post Office Address
Nome per intero di un eventuale sesto co-inventore	Full name of sixth joint inventor, if any
Firma del Sesto Inventore Data	Sixth inventor's signature Date
Residenza	Residence
Cittadinanza	Citizenship
Recapito postale	Post Office Address

(Si prega di fornire simili informazioni e firme peril terzo e gli eventuali ulteriori co-inventori.)

(Supply similar information and signature for third and subsequent joint inventors.)